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TITLE: Construction of a Vesicular Stomatitis virus Expressing Both a Fusogenic Glycoprotein and IL-12: A Novel Vector for Prostate Cancer Therapy

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14. ABSTRACT <p>Introduction: Vesicular Stomatitis Virus (VSV) infection of malignant cells results in oncolysis, sparing normal cells due to inherent differences in the interferon response pathway. In this study we explored enhancing VSV-G by engineering it to express the fusogenic glycoprotein from the Newcastle Disease Virus (VSV-F) to induce inter-cellular membrane fusion producing syncytia, which are incompatible with cell survival. Materials and Methods: Studies initially compared effects of VSV and VSV-F in vitro in prostate cancer cells lines, noting differential effects at different cell densities and the induction of apoptosis. Studies then compared effects of VSV vs VSV-F in a orthoptic mouse model of prostate cancer, focusing on tumor size at set time points and survival. Results: As the confluence of cells in the wells became greater, VSV-F showed more rapid cell kill than VSV-G (p<0.001). VSV-G mediated growth suppression by inducing apoptosis; this effect was slightly attenuated in VSV-F. In both single and serial viral injections VSV-F resulted in significant survival enhancement over control and VSV-G. Interestingly. Repetitive injections of VSV-G was no better than a single injection. Mechanistic studies suggested that some prostate cancer cell lines do not have as attenuated IFN response pathways, which can be over come by the high levels of IFN found within injected tumors. Discussion: Through the induction of the fusogenic protein, VSV-F maintain ns superior growth control of only moderately IFN responsive cell lines, most likely through an induced immune response.</p>					
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Table of Contents

Introductionpage 4

Body.....page 4

Key Research Accomplishments.....page 8

Reportable Outcomes.....page 8

Conclusions.....page 9

References.....page 9

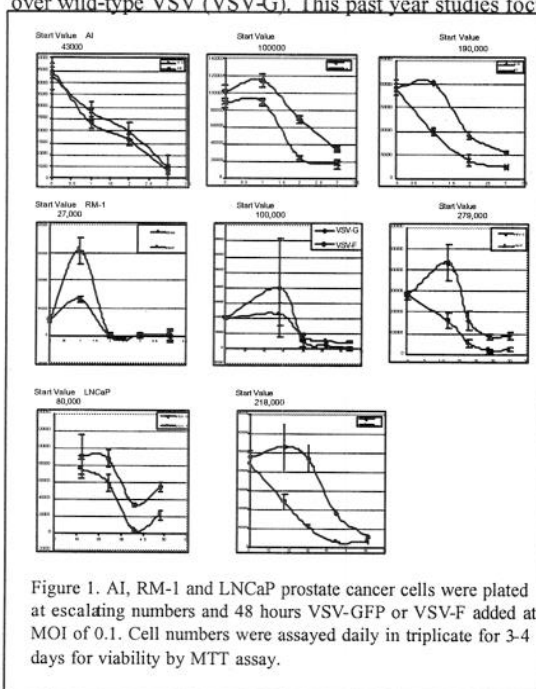
Introduction

The goal of this grant proposal is to examine the efficacy of an engineered VSV that expresses the fusogenic protein from the Newcastle Disease Virus (NDV) in comparison to wild type VSV. Fusogenic protein expression has been shown to lead to necrotic cell death through the production of large syncytia, which in turn may result in anti-tumor immune effects. Our efforts focused on a mutated fusogenic glycoprotein, L289a, which allowed for syncytia formation independent of the viral hemagglutinin-neuraminidase (HN) protein and exhibits a 50% enhancement in HN-dependent fusion over wild-type (wt) F protein. Through the introduction of syncytia formation, we proposed that we would engender better cell death in prostate cancer cell lines when compared to native VSV, while retaining its specificity for cancer cells and spare normal cells. Work during the first year demonstrated that both VSV and VSV-F in the presence of low doses of interferon did not kill normal prostate epithelial cells, while under the same conditions rapidly killed most prostate cancer cells in vitro. In each instance cell kill was seen even at low initial doses of virus and VSV-F demonstrated superior efficacy over wild-type VSV (VSV-G). This past year studies focused on completion of some in vitro studies and

exploration of the in vivo effects of VSV versus VSV-F.

Body

Earlier studies demonstrated enhanced ability of VSV-F to a variety of prostate cancer cell lines. We then explored some of the underlying mechanisms. First, we explored the effect of varying plating densities of cells prior to virus exposure on the killing abilities of both viruses. As shown in Figure 1, the superior abilities of



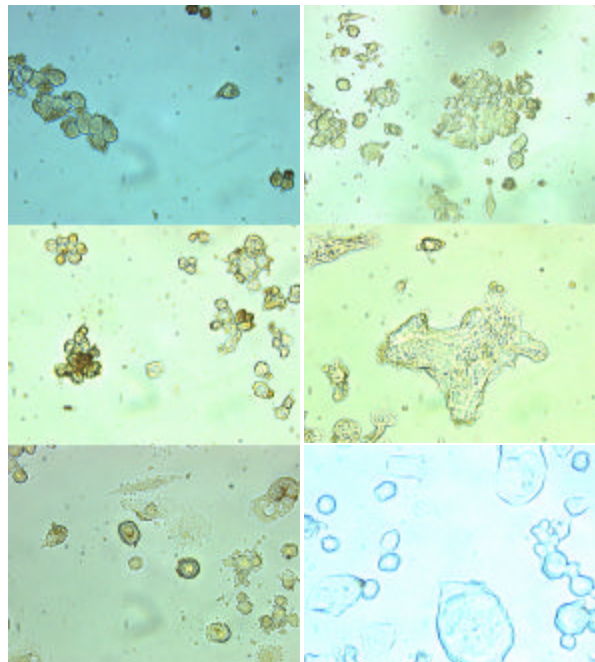
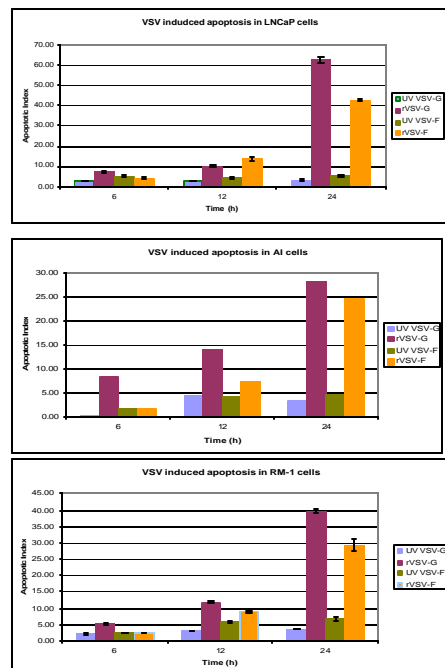


Figure 2. Plated LNCaP, AI and RM-1 cells were exposed to VSV-GFP, VSV-F or UV-inactivated VSV at MOI of 0.1. Apoptosis was determined by TUNEL assay at 6, 12 and 24 hours post-viral exposure in triplicate for each condition at each time point. Representative photomicrographs of each cell line are shown at 24 hours comparing VSV-GFP and VSV-F.

VSV-F were more apparent at the higher densities. This finding would support the notion that the enhanced effects of VSV-F are mediated through formation of syncytia which are more likely to form at higher cell densities. Published experience with VSV has demonstrated its ability to kill through induction of apoptosis. We studied whether or not this characteristic remained in the face of expression of the F protein and the induction of syncytia, which mediate necrotic cell death. As demonstrated in Figure 2a, there was marked induction of apoptosis mediated by both viruses in all 3 cell lines peaking at 24 hours post-virus exposure. In each instance VSV-G induced more apoptotic activity, though this was a modest difference. By light microscopy at 24 hours, VSV-F induced syncytia, a phenomenon not seen with VSV-G. TUNEL staining of these syncytia was negative (data not shown). Therefore, it would seem that VSV-F retains the powerful ability to induce apoptosis in addition to killing cells through syncytia formation.

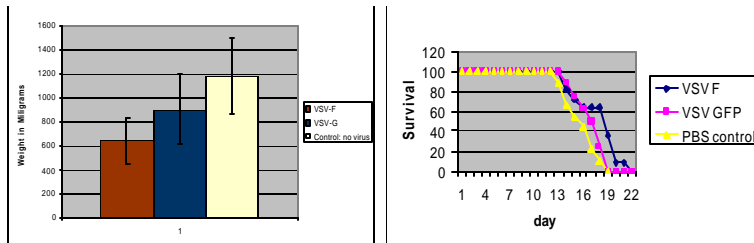


Figure 3. RM-1 orthotopic tumors were injected with VSV-GFP, VSV-F or PBS. Mice were either sacrificed at 14 days post-tumor cell inoculation or continued in a survival study. Tumors from sacrificed mice were weighed.

are inoculated into the dorsolateral prostate and 6 days later macroscopic tumors were injected with virus. The maximum liquid volume that can be injected into the mouse prostate without gross spillage is 50ul; this correlated with 8×10^8 vp which served as the dose tested. Mice were randomized to injection with a single therapeutic dose of VSV-G, VSV-F or equal volume of PBS. In the initial experiments all mice were sacrificed 8 days later, 14 days post-tumor inoculation, and wet weights of tumor obtained. Both vectors resulted in smaller tumors, 28% for VSV-G and 44% for VSV-F than control, respectively (Figure 3A) ($p=0.03$ VSV-G vs VSV-F t-test). As survival study was set up under the same conditions. Animals treated with VSV-F lived 25% longer than controls (Figure 3B) (19.5 ± 1 days vs 15 ± 0.5 days, $p < 0.001$, Mantel-Cox), while animals treated with VSV-G had median survival of 18 ± 0.45 days (VSV-G and VSV-F, $p=0.001$, Mantel-Cox). We then explored the potential benefit of repetitive injections of

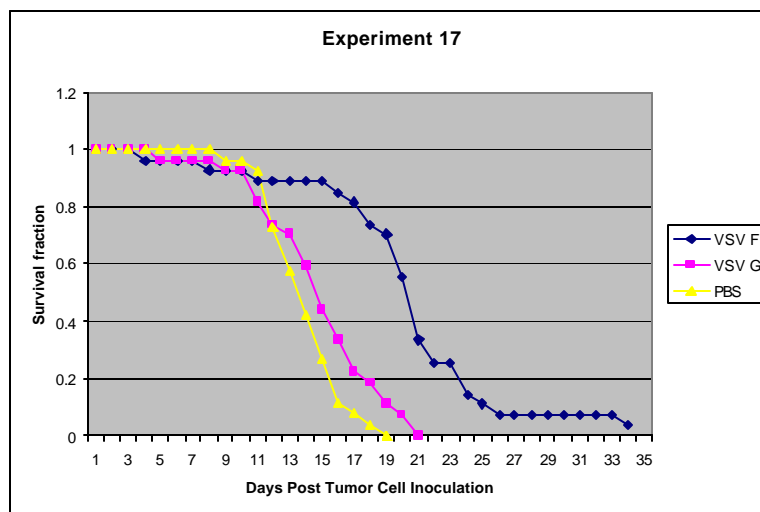


Figure 4. RM-1 Orthotopic tumors were injected with times with either VSV-GFP, VSV-F or PBS in a survival study.

Our attention then turned to the performance of in vivo studies, using the orthotopic RM-1 model. Tumor cells

VSV vectors. In a survival study mice were injected at days 6, 9 and 12 with 8×10^8 vp. Median survival for VSV-G was 16.2 ± 0.5 days compared to 14.3 ± 0.5 days (Figure 4) ($p=0.001$,

Mantel-Cox), compared to 23.6+/-2.3 days with 2 of 16 mice long term survivors.

Interestingly, we noted that there was no enhanced benefit to 3 injections of VSV over a single injection. Among the possibilities reviewed was that perhaps the defective interferon response pathways could be overcome at higher doses of IFN induced by the immune response against the vector to negatively impact viral replication. To address this hypothesis, we first ascertained levels of IFN- α in treated tumors on the third day following vector injection – the day on which in the repetitive injection study, the second dose of virus would be injected. Tumors were excised and weighed, flash frozen and mechanically lysed in the presence of protease inhibitors. The level of IFN- α was measured by ELISA with the calculation taking into account the weight of the tumor. Levels of cytokine in VSV-G treated

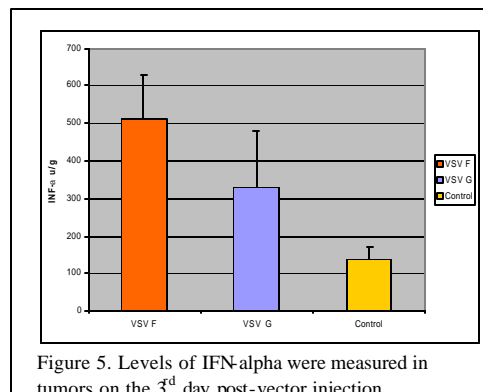


Figure 5. Levels of IFN-alpha were measured in tumors on the 3rd day post-vector injection.

tumors were double that of control while levels in VSV-F tumors were three times higher than control (Figure 5). To explore how IFN- α would impact on VSV replication, we exposed 3 cell lines, PC3, RM-1 and LNCaP at a fixed dose of VSV-G and VSV-F (0.1 MOI) and escalating doses of cytokine: 0-1000u (Figure 6). These studies noted differing responses to VSV infection:

PC3 cells are relatively resistant to either VSV virus, which can be blocked by low doses of IFN- α , reminiscent of how non-malignant cells react. In contrast LNCaP cells remain sensitive to VSV infection and replication even at very high doses of IFN- α , demonstrating the presence of fully defective IFN response pathways. RM-1 cells are sensitive to VSV at low doses of IFN- α , which can be overcome by higher doses of cytokine, implying only partially defective pathways. Indeed, at the levels of IFN- α measured within the tumor VSV-G is unable to replicate and kill RM-1 cells in vitro. However, VSV-F when injected serially is able to overcome the negative effects of IFN, inducing a significant enhancement in survival. Our hypothesis is that the induction of syncytia and its associated necrotic cell

death results in anti-tumor immunity which is augmented by the high levels of cytokine within the

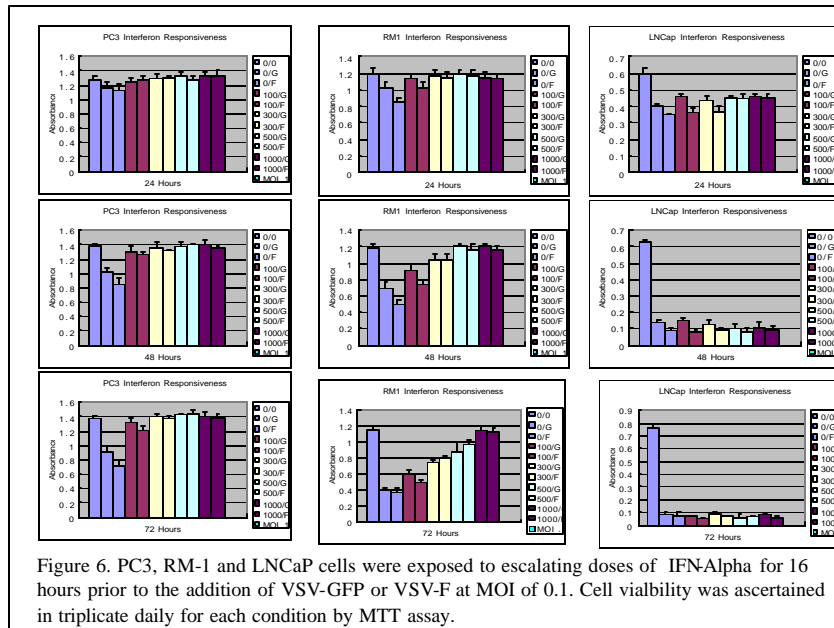


Figure 6. PC3, RM-1 and LNCap cells were exposed to escalating doses of IFN-Alpha for 16 hours prior to the addition of VSV-GFP or VSV-F at MOI of 0.1. Cell viability was ascertained in triplicate daily for each condition by MTT assay.

tumor. We are presently exploring the mechanisms mediating this effect and how it can be augmented by IL-12.

Key Research Accomplishments

1. The enhanced killing abilities of VSV-F are mediated through syncytia formation without significantly diminishing VSV's strong induction of apoptosis.
2. VSV-F results in superior growth effects over that of wild-type VSV, resulting enhanced survival in vivo.
3. Prostate cancer cells display differential IFN response pathways, which can adversely its oncolytic capabilities.
4. The growth suppressive activities of VSV-F occur at does of IFN which inhibit replication in IFN responsive cells.

Reportable Outcomes

Abstract at presented at the American Urological Association meeting Vesicular Stomatitis Virus as Oncolytic Viral Gene Therapy for Prostate Cancer. Seth A. Strope, Jian Pu, Sherwin Zargaroff, Savio LC Woo, Simon J Hall, Abstract # 429.

Conclusions

The data clearly demonstrate the enhanced abilities of VSV-F, especially with repetitive injections, in a cell line which has retained some interferon sensitivity. We are currently exploring the role of the immune response as the underlying driver of this activity.

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